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LYMPHOTROPIC VIRUSES IN ZAMBIA

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#### FOREWORD

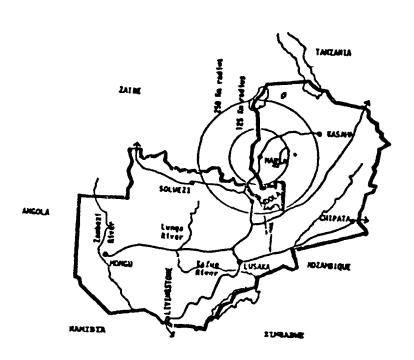
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#### Nonvenereal Transmission of HIV in Zambia



## Chronology 9/26/86 Funding approval 1/12/87 Initial community

1/12/87 Initial community seroprevalence survey 1/26/88 First community resurvey for HIV-1

2/12/89 Second community resurvey for HIV-1

6/30/90 HIV/HBV seroprevalence survey in antenatal women

12/21/90 Project completed

Nonvenereal transmission of HIV-1 can occur by transfusion of contaminated blood or blood products, including the sharing of needles and syringes by drug addicts. The possibility of transmission by blood sucking arthropods is theoretically possible (1,2) but unlikely (3). It has not yet, however, been studied in an environment where insect vectors of human diseases are highly prevalent.

We conducted a large seroprevalence study in the first quarter of 1987 for HIV-1 in Mansa, a community of about 50,000 people in Northeastern Zambia near the border with Zaire. In this community, transmission of P. falciparum malaria is intense and perennial. The hypotehsis tested was that prepubescent school children would have no evidence of HIV-1 infection unless they were born of MIV-1 infected mothers or had received contaminated blood transfusions. We also hypothesized that a random sample of households in Mansa would show that sexually active older children and adults would have proportionately increasing seroreactivity to HIV-1. Our hypothesis regarding HIV-1 transmission also predicted that if nonvenereal modes (contaminated needles and syringes, scarification, arthropods) were operative in Mansa, the age-specific prevalence rate for HIV-1 would parallel that for malaria and hepatitis B virus (HBV).

#### Methods

A total of 1,846 children of an estimated 8,000 children attending the 7 primary schools in Mansa were examined and had their serum collected and tested for HIV-1 antibody. The estimated number of children in Zambia under age 15 is 49% of the total population, and under age 5, about 37% (4). On this basis, the sample of school-aged children represented about 30% of the Mansa children between 5 and 14 years of age. Sera reactive in the screening ELISA (Abbott

Laboratories, North Chicago, IL) were confirmed by Western blot analysis (Dupont, Wilmington, DE) and/or ELISA using HIV-1 recombinant gp 41 and 120 peptides (Cambridge Bioscience, Cambridge, MA).

Blood films were collected at random from 84 school children for examination for malaria parasites. Antibody to the circumsporozoite (CSP) antigen of P. falciparum was also measured in these patients (5).

Using a cluster-sampling technique (6), a total of 222 Mansa households were randomly selected and all available members of each household had their serum collected and tested for HIV-1. The families of HIV seropositive school children, 222 randomly selected HIV-seronegative school children, and all households containing one or more HIV-seropositive school children were re-examined and tested for HIV-antibody at yearly intervals for 2 years.

Sera collected in the initial household survey sera also tested for HBV surface antigen (HBsAg), the infectious HBe Dane particle, and HBV core antibody (HCcAb) by ELISA (Abbott, North Chicago, IL). Children under 6 years of age who were seropositive for HBsAg were also tested for IgM anti-HBV core antibody (Abbott Laboratories, North Chicago, IL).

In a separate study conducted at the Mansa General hospital in July, 1990, 50 women in labor and their newborn infants were tested for HIV and HBV antibodies to investigate the potential for perinatal transmission of HBV in this community by determining the level of HBe antigeneria in 50 partuent women. The seroprevalence of parenterally transmitted HCV (non-A, non-B like) hepatitis was also determined.

#### Results

Of the 887 school boys age 6-14 years tested for HIV-1 antibodies, only one (0.1%) was seropositive by ELISA and confirmed by Western blot (Table 1). Five (0.52%) of 959 girls tested were reactive by ELISA and confirmed by Western blot

and/or recombinant antigen ELISA. Four of the 6 seropositive school children had indefiable risk factors for HIV-1 infection; two had mothers who were HIV+ and were probably infected at the time of their birth and two had received multiple blood transfusions for sickle cell anemia (Table 2).

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None of the HIV-1 seropositive children manifested signs or symptoms of AIDS. Almost all had a recent history of fever ("malaria") and one-half had splenomegaly. Most were anemic and underweight for their height, as were the majority of their classmates. Like their schoolmates, all HIV-1 infected children had received childhood immunizations and 4 had scarifications of their skin in the context of traditional medical and ritual practices. Among the HIV-seronegative school children, 55% of the boys and 59% of the girls had similar scarification on their face and/or trunk, and 5.7% of the boys and 6.4% of the girls had received blood transfusions for either sickle cell anemia or for severe malaria. As expected, 8 of the 84 blood films taken from randomly selected students were positive for P. falciparum, and 38 (43.2%) had high titers of antibody to the CSP antigen of P. falciparum.

During the first year of followup, one of 119 HIV-seronegative school girls and one of 101 seronegative school boys seroconverted to HIV; both acknowledged sexual exposure with persons who could not be tested. Two additional school boys, 12 and 15 years of age, seroconverted to HIV during the second year of followup; both had received blood transfusions in 1988 and 1987, respectively and seroconverted within 18 months of their transfusions. The overall two year incident of HIV was 4/220 (1.8%).

Among the 222 randomly selected households in Mansa, 47 (20.8%) had one or more members infected with HIV for a total of 92 (9.9%) serologically confirmed seropositives out of 924 persons tested. Twentyfour (6.9%) of the 348 males and 68 (11.8%) of the 576 females were infected. Proportionately, more females than

males were infected up to 30 years of age; therefore, males predominated (Figure 1). The average age of the adult men (>15 years) tested was 35.1 years with a median age of 32; the corresponding figures for women were 26.1 and 24 years, respectively.

Only 9 (2.5%) of the 358 children 15 years of age or younger in the initial household survey were infected with HIV-1 and no child between the ages of 4 and 11 years was infected. The 4 infected infants were all male and ranged in age from 8 months to 3 years; all were born of HIV-1 seropositive mothers. Three of the 5 HIV-1 infected 15 year olds admitted sexual exposure. Nineteen (8.4%) of the 226 households contained more than one HIV-1 seropositive member accounting for 62 (67.2%) of the 92 seropositive persons, which represents a highly significant clustering of HIV-1 cases (P=0.001).

Twelve of the 24 seropositive men in the initial household survey had a history of sexually transmitted disease(s), a significantly different percentage compared to the 13 (19%) of 68 seropositive women (P=0.01). In contrast, only 1 (4.2%) of the 24 seropositive men had received a blood transfusion; compared to 17 (25%) of infected females (P<0.05).

The Mansa household survey sera were also tested for antibody to HIV-2 and HTLV-I. None of the 325 sera tested positive for HIV-2, and only 2 (%) of 200 were positive for HTLV-1.

To determine the annual incidence of HIV infection, 283 HIV seronegative persons residing in 65 of the 226 Mansa households, including the 47 houses containing one or more infected persons sampled in 1987, were re-examined and serologically tested for HIV in 1988 and 1989. Seven incident cases were identified in 1988, and 9 incident cases in 1989 for a rate of 3.1% and 3.4%, respectively (Table 3). All of the incident cases occurred in households in which at least one other member was infected with HIV.

Forty-three (57.3%) of 75 children under 6 years of age in the Mansa household survey had either HBsAg or HBcAb. The prevalence of HBsAg progressively declined from a high of 45.3% of those under 6 years of age to a low of 13.3% among persons 36-40 years of age, when the prevalence of HBcAb increased from 20% of those under 6 years to over 70% of those 41 years or older (Figure 2). The prevalence of HBV markers in Mansa was significantly greater than in age and sex-matched urban patients attending the STD clinic at University Teaching Hospital (UTH) in Lusaka (data not shown). No doubt HBV causes morbidity and premature mortality from postnecrotic cirrhosis and hepatocellular carcinoma in Mansa, both of which are frequently encountered at the Mansa hospital.

The age-specific seroprevalence of HIV-1 and HBV infection (as measured by HBcAb) in Mansa approximates that which was hypothesized except that exposure to HBV occurs at birth or shortly thereafter in about 66% of Mansa children. The prevalence of HIV-1 is low during the first 5 years of life and almost always represents congenital/perinatal infection transmission or by blood transfusion.

#### Discussion

This study is one of two large-scale, community-based, prospective studies of the epidemiology of HIV-1 in Africa. Zilambi et al. (7) studied a historical cohort of people living in Yambuku in the remote of the equator providence of northeastern Zaire where an epidemic of Ebola-Hemorrhagic fever occurred in 1976. HIV-1 serologic tests in 1985 on stored sera collected during this epidemic showed that 5 of 659 residents had anti-HIV-1 antibodies; 3 of the 5 subsequently died of AIDS-like illnesses, but two were alive and healthy in 1985. None of 90 HIV-1 seronegative patients in 1976 had seroconverted by 1985, and only 3 of 388 cluster sampled persons had anti-HIV antibodies in 1985.

The comparative low but stable prevalence of HIV infection and its limited transmission in rural northeastern Zaire is in marked contrast to that seen in Mansa, Zambia.

When exactly HIV-1 first appeared in Mansa is not known. The first clinical cases of immunodeficiency such as aggressive atypical Kaposi's sarcoma were recognized after 1980. HIV-1 has reached a level of prevalence in Mansa comparable to that in Lusaka, Zambia. Mansa differs from Yambuku, Zaire in that it is more developed and is connected by a modern highway with other urban centers in northern Zambia and southern Zaire. There is considerable movement of people back and forth across the Zaire-Zambian border.

The most notable finding of this study is the rarity of HIV-1 infections in Mansa among adolescent school-aged children, the majority of whom had antibodies to P. falciparum malaria. This observation does not exclude transmission of HIV-1 by insect vectors, but it does suggest that if this occurs, it does so at a very low frequency. The nigh prevalence of HBV infection in Mansa residents probably reflects a high frequency of maternal perinatal transmission. It is also likely that scarification of the skin with unsterilized instruments such as razors, which begins early in life, also transmits HBV. The instrument preferred for scarification is an unsterilized razor blade which is also used by traditional healers to administer "medicaments." Adolescent children in Mansa between 5 and 12 years of age had a remarkable low prevalence of HIV antibody and most were in good health. At least one healthy seropositive 5 year old boy had no discernible risk factors for HIV-1, and he could represent the type of indigenous infection described in Yambuku, Zaire (7).

The remarkable clustering of HIV-1 cases in Mansa most likely reflects both heterosexual and perinatal transmission. Although 12 children were identified as HIV-infected in the household surveys, all were under 6 years of age. None

of the children between 6-14 years of age residing in households containing at least one infected adult were infected with HIV. This also indicates that frequent nonventeal household contact does not transmit HIV.

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The rarity of HIV-1 among school children and the clustering of adult infections to a small number of households in Mansa should facilitate HIV-intervention strategies. The risk of sexual transmission among sexual partners is significant and apparent, and should be lessened by screening of all blood donors, sexual counselling of school children, and instructing adults about the availability and proper use of condoms.

#### Addendum

A one year extension of this project was requested to further explore the transmission of HBV in Mansa. We were particularly interested in the epidemilogic relationships been HBV and postnecrotic cirrhosis and hepatocellular carcinoma and whether hepatitis C virus (HEV) was prevalent in the community. It was not clear whether the high prevalence of HBV reflected perinatal transmission of the virus or its acquisition by various parenteral exposures (infections, scarification, transfusions) which begin early in life.

We studied 50 post partum mothers and their newborn infants delivered at the Mansa Hospital from 25 June to 4 July 1990. This 500-bed hospital serves as the primary health care facility for Mansa, Zambia and as the referral hospital for Luapula Province. The majority of mothers in the Mansa area deliver on the hospital's maternity ward.

Informed consent was obtained from all study mothers. Blood samples were collected without complications by venipuncture from all mothers prior to delivery, and from the antecubital or femoral vein of their babies within 12

hours after birth before any blood transfusions or invasive procedures. The maternal medical record was reviewed, and each mother was interviewed using translators when necessary to obtain demographic data, data on prenatal variables (weight gain, gestation, complications), prior blood transfusions, medications prescribed, and current or prior medical diagnoses, including jaundice and HIV infection.

To determine the prevalence of hepatitis B and C markers, sera were tested for antibodies to HBV surface antigen (HBsAq); HBV e antigen (HBeAq); and to HBV core (anti-HBc), (Abbott Laboratories, North Chicago, IL) and HCV (anti-HCV) antigens by ELISA and recombinant immunoblot assay (RIBA), (Ortho Diagnostic Systems, Raritan, NJ). Serum samples were analyzed at the University Hospital laboratory in Lusaka, Zambia, for hepatitits B markers, syphilis (Rapid Plasma Reagin) and for HIV-1 antibodies by ELISA (Recombigen R-HIV EIA, Cambridge BioScience, Worchester, MA), with seropositive sera confirmed by Western blot analysis (Dupont, Wilmington, DE). All serum samples were refrigerated at 2 to 8 degrees C or frozen while awaiting analysis in Zambia and during transport to Washington, D.C., where tests for anti-HCV were performed. Women seropositive for HBeAq were considered to have replicative HBV infections capable of causing prenatal transmission. Possible differences in the prevalences of the serological markers between the mothers and their babies were analyzed using McNemar's test for paired samples. Transfusion and scarification exposures as well as abnormal physical findings were determined and correlated with serclogical results using the ordinary chi-square test for independent proportions, and McNemar's chi-square test for correlated (paired) proportions, using a 0.05 level of significance. Demographic characteristics of the total cohort were compared with those of mothers/babies who were seropositive for HBsAg, anti-HBc and anti-HCV, using the Student's t test.

During the study period, all mothers presenting consecutively to the maternity ward in acitve labor consented to be included in the study. Three of the mothers entered into the study did not deliver during the study period. Two mothers delivered fraternal twins and were counted as two mother-baby pairs for statistical analysis. Although sera were obtained from all 49 babies born during the study period, several samples were of insufficinet volume for all hepatitis serology testing.

Analysis of maternal and neonatal characteristics revealed no significant differences in age, maternal weight gain, hemoglobin concentrations, length of gestation or neonatal birth weight between the total cohort, and mothers and/or neonates who were seropositive for HIV-1,, HBsAg, antiOHBc and antiOHCV (Table 3). HBsAg was detected in 3/49 (6%) of the maternal samples and 0/44 of the baby samples. Two of the three HBsAg-positive mothers also had HBeAg.

Excluding nontested baby sera, chi-square analysis of mother-baby pairs using McNemar's test showed similar prevalence of anti-HBc in mothers and babies; 38/49 mothers (77.6%) and 30/39 (76.9%) of their babies were positive for anti-HBc (Table 4). As expected, analysis of 37 mother-baby pairs using McNemar's test showed similar prevalence of anti-HBc in both (p> 0.05). A maternal history of blood transfusions and/or scarification procedures was not significantly associated with maternal or baby anti-HBc seropositivity, nor with the maternal prevalence of HCV.

The anti-HCV assay was positive in 11/49 (22.4%) of the mothers and 5/46 (10.9%) of the babies. The HCV RIBA was positive in 6 of the 11 (54.5%) anti-HCV-positive mothers, nagative in 3 and indeterminant in 2. A statistically significant association by chi-square analysis was found between anti-HCV (as determined only by ELISA) and anemia (hemoglobin <10 g%) in HCV seropositive mothers (p<0.05); however, no significant association was found

between anti-HCV, HBsAg, or HBeAg markers in mothers or babies. A maternal history of blood transfusions and scarifications procedures was not significantly associated with either maternal or baby anti-HCV seropositivity.

Eleven (22%) of the mothers were Western blot-confirmed seropositive for HIV-1, including two of 11 who were infected with HCV (RR=1.14). Maternal HIV infection was strongly associated with a history of blood transfusion ( $X^2=2.66$ , p=0.008). There were no significant associations between maternal infection with HIV and a history of skin scarification ( $X^2$ .11, p= -.37). The RPR test for syphilis was negative in all mothers.

HBV is highly prevalent throughout most of subsaharan Africa. Infection by HBV is thought to occur in Africa in early childhood by nonvenereal contact with skin lesions and secretions of HBeAg-positive household members (8,9). This type of horizontal transmission is most likely the case in Zambia, since only 2 of the 50 antenatal women studied had replicative HBV infections and were thus likely to transmit this virus to their offspring perinatally. By contrast, the high prevalence of HBV in Asia is attributed to vertical (perinatal) transmission from HBeAg-positive mothers (10). Clustering of HBsAg carriers in small communities and within households in Africa has also been attributed to bites from HBeAg-positive bedbugs infesting the bedding of young children (8). The potential for transmission of HBV and HCV by bedbugs in Mansa is under study.

The high prevalence of anti-HBc in Mansa mothers indicates the significant level of exposure of the population to HBV, most likely by close household and/or sexual contact. Babies infected by HBeAg-positive mothers during the perinatal period usually do not become seropositive for HBsAg until 3 to 6 months of age (10). Additional studies correlating exposure variables in Mansa

children with hBsAg and anti-HBc seroconversion should be useful in determining the timing and primary modes of transmission of these hepatitis viruses during infancy and early childhood.

The exact meaning of positive anti-HCV tests isn not well understood. The recently introduced HCV ELISA and RIBA tests use the same recombinant gene product as the test antigen; however, it is unclear at present if the RIBA assay is a more specific test for the virus than the ELISA, or if one of the assays is more sensitive than the other. At least one study suggests that most anti-HCV-positive patients with persistent hepatitis are at risk for the development of hepatocellular carcinoma (11). The high prevalence of anti-HCV relative to HBsAg seropositivity in the Mansa mothers and their infants suggests that HCV may also be a significant etiologic factor for hepatocellular carcinoma in this population, as has been reported from South Africa (12).

The statistically significant association between clinical anema (hemoglobin <10g%) in pregnant women and anti-HCV in this study is difficult to explain, but could reflect suppression of erythrocyte production by the chronic liver inflammation caused by HCV. Although prior studies have demonstrated the transmission of HCV through blood products, the small sample size in this study does not demonstrate any statistically significant association between anti-HCV seropositivity and exposure to transfusions and scarification procedures.

Additional seroprevalence studies of Zambian blood supplies, randomly-selected households, and high-risk individuals would be useful in identyfying the potential for transmission of the infection through blood transfusions, sexual intercourse or other modes. Public health measures, now primarily concerned with control of HIV-1 and hepatitis B transmission, require expansion when the precise modes of HCV transmission are determined and high-risk populations are defined. The effect of coinfection of HIV-1 on HBV and/or HCV and whether or not they share the same risk factors also require forther study (13).

#### Appendix A

#### Scientific Publications Supported by G18796

- 1. Hira SK, Wadhawan D, Kamanga J, Kavindele D, Macuacua R, Patel PS, Ansary MA, Macher AM, Perine PL. Cutaneous manifestations of human immunodeficiency virus in Lusaka, Zambia. J. A. Acad. Dermatol. 1988; 19:451-57.
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Table 1

Age and sex specific seroprevalence of HIV-1 among school children in Mansa, Zambia

	Males		Females	
Age(yrs)	Tested	NO HIV-1(%)	Tested	HIV-1(%)
6	10	1	15	0
7	129	0	165	0
8	192	0	200	1(0.5%)
9	140	0	145	2(1.38%)
10	140	0	161	1(0.62%)
11	110	0	151	1(0.66%)
12	149	0	112	0
13	13	0	9	0
14	2	0	1	0
15	2	0	0	0
Total	887	1(0.11%)	959	5(0.52%)
	_	-		

Table 2
Status of HIV-infected school children in Mansa

Case No.	
LM2343	A 6 y/o boy in good health with no history of blood transfusions; no other risk factors identified. His mother was seronegative for HIV antibody.
LM724	A 11 y/o girl in apparent good health whose mother and younger sibling, the last born child, were also seropositive for HIV.
LM840	A 9 y/o girl with sickle cell anemia who had received multiple blood transfusions. Her mother and two sisters wre seronegative for HIV.
LM854	A 9 y/o girl with sickle cell anemia and a history of multiple blood transfusions.
LM904	A 9 y/o girl with scarifications and the last born in her family. Her mother was seropositive for HIV but her older siblings were seronagative for HIV.
LM1280	A 10 y/o girl with no risk factors for HIV. Her mother and siblings were unavailable for testing.

Table 3: Characteristics of 50 Antepartum Women Admitted to the Labor Wards of Mansa General Hospital

	Total			anti-	anti-HCV(+)	
Feature	Sample n = 50	HBsAg(+) n = 3	anti-HBc(+) n = 38	$ELISA \\ n = 11$	RIBA n = 6	HIV(+) n = 11
Age (years)	24.4 ± 5.9	21.7 ± 4.2	24.9 ± 5.9	22.7 ± 6.0	25.0 ± 7.4	23.5 ± 5.6
Weight gain (kg)	4.4 ± 3.2	5.7 ± 3.0	4.2 ± 3.5	5.2 ± 2.7	6.5 ± 2.5	3.98 ± 3.1
Hemoglobin (9%)	$12.3 \pm 1.8$	12.0 ± 0.9	12.5 ± 3.6	11.1 ± 2.7*	11.2 ± 3.2	11.7 ± 2.2
Gestation (wks)	37.7 ± 4.2	40.2 ± 3.9	38.3 ± 3.6	36,8 ± 3,3	36.3 ± 3.6	39.1 ± 3.3
Birth weights	2.98 ± 0.5	1	2.94 ± 0.45	3.23 ± 0.21	ı	3.08 ± 0.4

Table 4: Prevalence of HIV-1 and Hepatitis B and C Vicuses in Mothers and Their Newborns at Mansa General Hospital

	Æsag (+)	anti-HBc (+)	anti-HCV (+)		
			ELISA	RIBA	
Mothers	3/50(6%)*	38/49(76%)	11/49(22.4%)	6/11(54.5%)	
Neonates	0/44	30/39(76.9%)	5/46(10.9%)	-	

<sup>\*2/3</sup> were HBeAg positive